

Experiment 4

Effect of pH on Ibuprofen Solubility

Exercises to be completed BEFORE coming to the laboratory:

1. Summarize the safety information and precautions required for this experiment in your lab book;
2. Write a short paragraph summarizing the objectives of the experiment.
3. Ibuprofen is a weak acid, write an equation to show how a weak acid exists in aqueous solution. What is the relationship between pH of an aqueous solution of a weak acid and its pKa?
4. What is the solubility of ibuprofen according to the British Pharmacopeia? Why is ibuprofen not very soluble in water?
5. What is the major form of ibuprofen that exists in solutions of pH 11 and pH 2?
Comment on the expected solubility of ibuprofen in solutions at these pH values.

Chemicals used

Phosphate Buffer (pH = 7, 0.1 molL⁻¹ NaH₂PO₄ and Na₂PO₄) <i>Description:</i> colourless solution <i>Risk:</i> harmful if swallowed <i>Precautions:</i> avoid contact with eyes <i>Disposal:</i> solution to drain, flushed well with water	Sodium Hydroxide Solution (5%) <i>Description:</i> colourless solution <i>Risk:</i> harmful if swallowed <i>Precautions:</i> avoid contact with skin and eyes. <i>Disposal:</i> solution to drain, flushed well with water
Ibuprofen <i>Description:</i> white powder <i>Risk:</i> Not classified as hazardous <i>Precautions:</i> avoid contact with eyes <i>Disposal:</i> solution adjusted to pH between 6 and 10 to drain, flushed well with water	Hydrochloric acid (1%) <i>Description:</i> colourless solution <i>Risk:</i> harmful if swallowed <i>Precautions:</i> avoid contact with eyes; rinse immediately with water <i>Disposal:</i> solution to drain, flushed well with water

Total time: 3 hours
Work in pairs

1. Introduction

This experiment aims to study and relate solubilities of drugs to their acid-base chemistry. The solubility of ibuprofen (Figure 1) will be investigated to understand the issues involved with making an aqueous solution of this drug.

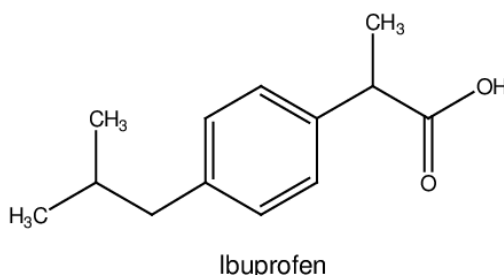


Figure 1: Molecular structure of ibuprofen

The effect of pH on the solubility of ibuprofen, which is a weak acid, will be examined. Ibuprofen will be dissolved in water at high pH, then pH will be slowly lowered and ibuprofen precipitation will be monitored by absorbance spectroscopy. During the slow reduction in pH the changing concentration of dissolved ibuprofen in the sample will be determined. Additionally, the pK_a of ibuprofen will be experimentally determined.

2. Theory

Regardless of a drug is administered, whether orally, instilled into the eye, ear or nose, injected or applied externally to the skin, the therapeutic effect will take place much more rapidly if the drug is already in solution.

Many drugs are either weak acids (e.g. aspirin, ibuprofen) or weak bases (e.g. ephedrine, procaine). In general, the un-ionized forms of these drugs are less soluble in water than are the ionized forms. For example, aspirin as the free acid is only soluble 1 part in 300 parts in water by weight, but aspirin as the anion (salt) is soluble 1 part in 6 parts in water by weight. Since the pH of the solution determines the fractions of drug in the ionized and unionized forms, the solubilities of weak acids, weak bases and their salts are very dependent on solution pH. For a weak acid, this effect is expressed mathematically by Eq. (1), derived from the Henderson-Hasselbalch equation.

$$\text{pH} = \text{pK}_a + \log \frac{S - S_0}{S_0} \quad (1)$$

S_0 is the solubility of the un-ionized form of the drug in water and S is the observed solubility of the drug at the pH of the solution ($S = S_0 + [A^-]$, where $[A^-]$ is the concentration of the ionised drug in solution).

The analogous equation for a weak base is expressed in Eq. (2)

$$\text{pH} = \text{pK}_a - \log \frac{S - S_0}{S_0} \quad (2)$$

S_0 is the solubility of the weak base in water, and K_a is the dissociation constant of its conjugate acid.

These equations are useful for formulation purposes, since they enable prediction of the pH required for complete drug dissolution and estimation of the solubility of a drug at any particular pH. When other drugs are added to an established formulation the pH might change, leading to precipitation of the drug. This is a physical incompatibility. Equations (1) and (2) allow quantitative prediction of the effect of adding, for example, an acidic drug to a solution of a salt of a weak acid as well as allowing for an estimation of a drug's pK_a .

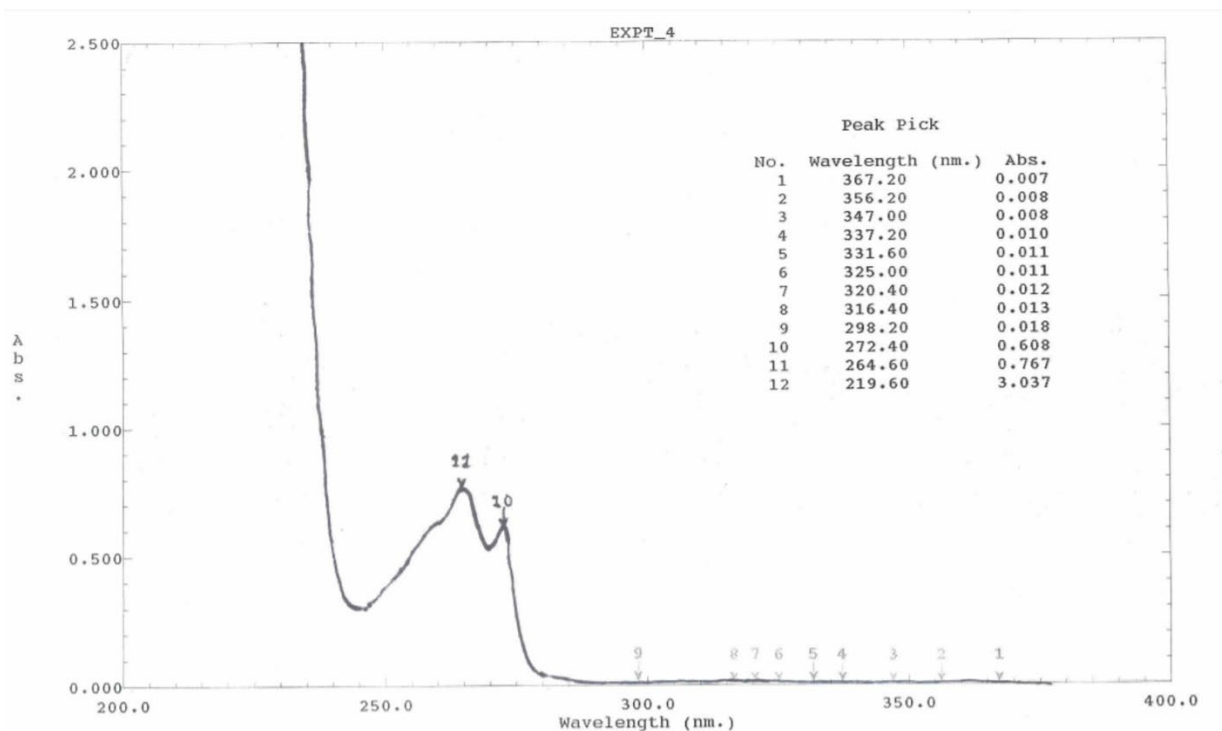


Figure 2: UV-Vis spectrum of ibuprofen (0.5 mg mL^{-1} , pH 7 phosphate buffer). Peak 11 shows the λ_{max} .

It can be seen from Figure 2 that ibuprofen has maximum wavelength of absorption at ~264 nm. This absorption maximum will be used in this experiment to measure the absorption of all the prepared standards and samples.

3. Experiment

You will initially be divided into pairs, each person in the pair will tackle a different section of the experiment. If the experiment is not completed on time the groups will share data so that everyone can complete the lab report. You should endeavor to keep your laboratory book recording as simple and legible as possible. Data may be shared by either copying or photocopying each other's data. Tables are shown throughout this document that can be used as templates for recording experimental data in your laboratory book.

Group 1

3.1. Preparation of ibuprofen stock solution

1. Accurately weigh ~250 mg of the solid ibuprofen and quantitatively transfer it to the 50 mL volumetric flask labeled 250 mg/50 mL ibuprofen. **Record the exact mass of the ibuprofen in your lab book.**
2. Add ~30 mL of pH 7 buffer solution. Shake to dissolve and place flask in the sonic bath. When the solids are mostly dissolved, make up to the mark with pH 7 buffer and sonicate the solution for a further 5 minutes.
3. Pour 25 mL of the ibuprofen solution into each of the two centrifuge tubes, making sure that the two tubes are the same weight and spin at 4000 rpm for 5 minutes. **Be careful not to bump your flasks when removing them from the centrifuge.**

3.2. Preparation of ibuprofen standards

Four **10mL** volumetric flasks are labeled as 0.25, 0.50, 1.00 and 1.50 mg mL⁻¹.

1. Add volumes of the (given in Table 1 below) to the 4 flasks using an automatic pipette.

Carefully pipette the supernatant of the centrifuged stock solution so that you don't transfer any undissolved solids to the standard solutions.

2. Make each flask up to the mark with pH 7 buffer. Invert the flasks a few times before taking samples from them. **Be sure to record the uncertainty values for each flask.**

Volume of IBUPROFEN STOCK (mL)	Label on 10 mL volumetric flask	Uncertainty of flask ($\pm$ mL)
0.5	$\approx 0.25 \text{ mg mL}^{-1}$	
1.0	$\approx 0.50 \text{ mg mL}^{-1}$	
2.0	$\approx 1.00 \text{ mg mL}^{-1}$	
3.0	$\approx 1.50 \text{ mg mL}^{-1}$	

3.3. Preparation of Nurofen tablet samples

1. **Make a note of the label claim for mass of ibuprofen in the Nurofen tablets in your lab book**
2. Take 2 NURAFEN tablets and grind them separately in mortar and pestle.
3. Quantitatively transfer each tablet to separate labeled 250 mL volumetric flasks. **Record the exact mass in your lab book.**
4. Add 80 mL of pH 7 buffer and shake to dissolve. Sonicate these flasks for 5 minutes.
5. Binder material in the tablet will be insoluble so when it looks as if most of the tablet solid is in solution, fill each flask to the mark with pH 7 buffer.
6. For each tablet sample, pour 40 mL into TWO 50mL centrifuge tubes ensuring that the two tubes for each sample are balanced in weight. Note there will be 4 tubes total.
7. Place the tubes from each tablet in the centrifuge and spin at 4000 rpm for 5 minutes. **Be careful not to bump your flasks when removing them from the centrifuge.**

3.4. Measurement of the ibuprofen standards and Nurofen tablet samples

Absorbance measurements of the standards are carried out using the QUANTITATIVE Mode of the Shimadzu UV-vis spectrophotometer.

- Click the 'Acquire mode' menu button and make sure that 'Quantitative' is checked.
 - Input the λ_{max} in the wavelength parameter box
 - Number of cells = 1
 - Input the concentration range for your standards
1. Insert two quartz cuvettes containing type-1 water and press auto zero – notice that the absorbance reading has adjusted to zero.
 2. Replace the solution into the cuvette closest to you with the lowest concentration standard and press READ. Enter the concentration of the solution you are measuring. This will place the absorbance reading into a table.

It is important to wash the sample cell several times with Type-1 water and then rinse with the sample to be measured.

3. Your other solutions can be measured by simply replacing the sample solution and pressing READ. Leave the blank as it is.
4. When measuring the ibuprofen tablet samples (or pH adjusted samples) press 'UNKNOWN'. This will add the absorbance readings to the table with concentration as 0.

After all measurements are finished click on the 'Manipulate' menu and press 'data print' to obtain a printout of your data. **Label the printout clearly to indicate what the unknown samples are as this will be copied for other students if necessary.**

Alternatively, you can clearly record all measurements in your lab book, a table such as the one below would be appropriate.

Label on 10 mL volumetric flask	Absorbance at λ_{max}
$\approx 0.25 \text{ mg mL}^{-1}$	
$\approx 0.50 \text{ mg mL}^{-1}$	
$\approx 1.00 \text{ mg mL}^{-1}$	
$\approx 1.50 \text{ mg mL}^{-1}$	
Nurofen tablet 1	
Nurofen tablet 2	

Group 2

3.5. Calibrating the pH meter

To calibrate the Sartorius pH meter

1. Rinse the electrode with water and blot it dry with some tissue paper
2. Place the electrode in pH 4 buffer solution (pink)
3. Press the 'Standardize' button. The display will show a flashing "S" and a picture of the electrode. When the calibration is finished the picture of the electrode will disappear and the display will show a % slope and asymmetry values.
4. Rinse the electrode again and blot dry with tissue.
5. Place the electrode in the pH 9.2 buffer solution (colourless)
6. Press the 'Standardize' button as described in instruction point 3
7. When the calibration is finished the electrode picture will disappear and the display will show a % slope and asymmetry values along with the two pH values that it has been calibrated with. If this does not occur see a demonstrator.

3.6. Preparing ibuprofen solutions at a range of pH values.

1. Take a 150 mL beaker to an analytical (4 decimal place) balance and weigh ~200 mg of the solid ibuprofen into the beaker. **Record the exact weight into your lab book.**
2. Add 80 mL of Type-1 water and a magnetic stirrer bar. Place the beaker on the magnetic stirrer. Add 5% NaOH drop-wise to bring pH up to 11. Stir for at least two minutes. If pH drops, add more NaOH until pH stabilizes at 11.
3. Transfer the solution to a 100 mL volumetric flask and fill it to the mark with Type-1 water.
4. Transfer this solution back to the 150 mL beaker previously used. **Measure the pH and record it.**
5. Transfer a 5 mL sample to a centrifuge tube **labelled 1**. Leave the beaker on the magnetic stirrer.
6. While monitoring the pH, add 1% HCl acid one drop at a time to the solution in the beaker. The magnetic stirrer should be stirring. Allow the pH to settle for about 30 seconds between drops.

7. Take 5 mL samples at pH ~ 7.5 , ~ 7.0 , ~ 6.0 , and then at six approximately evenly spaced points across the pH range 5.5 to 2.0. **Record the accurately measured pH values in a table like the one illustrated below.**

Label on centrifuge tube	Measured pH	Absorbance at λ_{max}
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

8. Centrifuge these samples for 10 minutes at 6500 rpm. **Be careful not to bump your flasks when removing them from the centrifuge.**

3.7. Measurement of ibuprofen absorbance at different pH values

Instructions for using the spectrometers are presented in Section 3.4 above.

Auto zero the spectrophotometer using type one water in both cuvettes.

1. Using a plastic dropper, transfer the supernatant sample to a quartz cuvette, starting with the sample with the highest pH (Type-1 water can be used as the blank).
2. Read the absorbance of each solution on the spectrophotometer at the wavelength of its maximum absorption. Wash the sample cell thoroughly with Type-1 water and then with the next sample to be measured in between measurements.

Clearly record all absorbance measurements in your lab book.

4. Report

Your lab report should start with a summary of the safety information and any precautions required for this lab, and then be divided into sections – corresponding to sections below. For each section summarize the objectives of the section and write an experimental procedure (third person, past tense description of how the experiment was performed), and then answer the questions for that section. You must provide one fully worked example for each of the calculations below including the calculation of uncertainty.

4.1. Results

1. Calculate the exact concentration of the ibuprofen stock solution and standards in mg mL^{-1} (with uncertainty), and report the corresponding absorbance measurements. Present these values in a table (template below)

Exact weight of IBUPROFEN = $\pm$ (g)
 Exact concentration of IBUPROFEN STOCK = (mg mL^{-1})

Volume of IBUPROFEN STOCK (mL)	Label on 10 mL volumetric flask	Exact concentration of Ibuprofen standard (mg mL^{-1})	Absorbance at λ_{max}
0.5	$\approx 0.25 \text{ mg mL}^{-1}$		
1.0	$\approx 0.50 \text{ mg mL}^{-1}$		
2.0	$\approx 1.00 \text{ mg mL}^{-1}$		
3.0	$\approx 1.50 \text{ mg mL}^{-1}$		

2. Prepare and present a calibration graph from the absorbencies of the standard ibuprofen solutions. The plot should be absorbance vs. concentration of ibuprofen/100 mL. Use appropriate software to obtain the equation of the line, a regression analysis and the R^2 value.
3. The equation for this calibration curve can be used to calculate the concentration of the drug in each tablet sample measured. Calculate the mass of ibuprofen in the Nurofen tablets you analysed today.
4. The equation for this calibration curve can also be used to calculate the concentration of the drug in each sample at different pH values. For samples taken when the ibuprofen was not completely dissolved, the concentration that you measure is the maximum concentration that could dissolve at that pH – this is the solubility (S). Report your measured pH, corresponding absorbance values, calculated ibuprofen concentrations and solubility (with uncertainty) in a table

5. Plot concentration of ibuprofen vs pH. Use the line graph option to connect your data points as this is not a linear graph. What is your estimate for S_0 (solubility of the un-ionized form of the drug) in mg/100 mL? You should be able to find S_0 from this plot of concentration versus pH.
- (e) Plot $\log \frac{(S - S_0)}{S_0}$ vs. pH. Use the line graph option to connect your data points as this is not a linear graph.

4.2. Questions

1. Use your concentration vs. pH plot to indicate where you expect ibuprofen to be 0.1, 1, 10, 50, 90, 99, and 99.9% ionized.
2. How does your S_0 compare with solubility of ibuprofen according to the British Pharmacopeia?
3. Explain the effect of pH on the solubility of ibuprofen using the information gained in the experiment. Be specific and demonstrate that you understand why the solubility changes with pH.
4. Estimate the pK_a of ibuprofen. Explain what it means, how you came to that value, and how your value compares to the pK_a for ibuprofen in the literature.
5. Compare the experimentally determined mass of ibuprofen in the tablets with the label claim.
6. Consider methods other than changing pH that might be used to increase solubility of drugs with low aqueous solubility.
7. Comment on the reliability of the calculated concentration of the ibuprofen stock solution used to make the calibration standards. Provide an experimental reasoning for your answer and suggest a way to improve the experiment so that the determination of ibuprofen mass in the tablet samples could be more accurate.

4. Reference

1. Harris, D. C. *Quantitative Chemical Analysis*, W. H. Freeman and Co.: New York, 7th ed. 2007. Sections 4.8, 6.3-6.6 and 18.1-18.5